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ENZYMATIC ISOLATION OF ADULT ONCHOCERCA VOLVULUS
AND ASSESSMENT OF MICROFILARIAL DENSITIES IN ETHANOL-FIXED MATERIAL¹

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1. INTRODUCTION

The isolation of living, undamaged adult Onchocerca volvulus from nodules can be achieved by enzymatic digestion of the surrounding human tissue using collagenase. As fresh material used to be essential for this procedure, problems of transport and long storage often prevented its being employed by those working in the field. For this reason the digestion of ethanol-fixed Onchocerca nodules was tried, and the feasibility of making a qualitative and quantitative evaluation of the adult worms so isolated was investigated.

Microfilariae in skin snips can also be quantitatively assessed following enzymatic digestion of human tissue with collagenase (Schulz-Key, 1978). This technique is recommended for special investigations in the laboratory when an exact assessment of microfilarial densities is needed, e.g. for drug trials. The collagenase technique has now also been adapted for assessment of microfilarial densities in ethanol-fixed biopsies. This method is of interest when immediate digestion of fresh material is not possible.

2. MATERIAL AND TECHNIQUES

Twenty-five nodules excised from 10 patients were fixed in a mixture of 70% ethanol and glycerine (10:1) and stored for four weeks. They were then washed several times in phosphate-buffered saline (PBS) for 24 hours, transferred to a 0.3% collagenase solution in PBS and treated as described by Schulz-Key et al. (1977). Small pieces of tissue cut from the capsule of the fixed nodules were also weighed and treated in the same way to assess the microfilarial densities in this tissue.

One hundred and eight pairs of closely neighbouring skin snips were taken with a Walser punch from 55 patients. They were weighed on a torsion balance and then placed in the wells of microtitration plates (Scheiber et al., 1976) either filled with normal saline (controls) or with a mixture of ethanol and glycerine (test).

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Microfilariae which emerged from the unfixed control snips or capsule biopsies were counted after 24 hours. The biopsies were then removed and digested with collagenase to assess the number of microfilariae remaining in them and hence the total number of microfilariae contained.

The ethanol-fixed biopsies were examined four weeks after fixation. The test biopsies were washed in PBS for 24 hours and then placed in two drops of a solution of 0.5% collagenase in PBS for 72 hours at 30°C. On the second day of digestion another drop of enzyme solution was added. To inhibit the growth of fungi in some of the wells, merthiolate was added (1:1000). This did not impair the activity of the enzyme. Before counting (microscope 60X) the fibres of the tissue had to be carefully pulled apart on the slide using dissecting needles.

3. RESULTS

The process of digesting was longer with fixed than with fresh material. However, a prolonged exposure to the enzyme solution for several days did not affect the morphology of the fixed worms or microfilariae by comparison with unfixed digested specimens.

Nodules

Usually worms could not be isolated from the nodules before the second day of incubation, but they were then in very good condition. A qualitative and quantitative examination of the parasites could be made as follows:

- (a) Assessment of intact worms (i.e. those reckoned to have been alive before fixation).
- (b) Assessment of degenerate worms and their calcified fragments.
- (c) Condition of the females with regard to the presence of developmental stages or empty uteri. As the fixed worms were less transparent than fresh ones the microscopical study needed more care. Some worms had to be cleared by further immersion in glycerine or lactophenol.
- (d) Quantitative assessment of the total number of developmental stages in the females as described by Schulz-Key et al. (1980). Even the differentiation of normal from pathologically-altered stages was possible, although sometimes this was more difficult owing to shrinking effects, especially of the egg-shell.

Microfilariae

On an average 78% of the microfilariae had left the unfixed control snips before digestion. Although previous investigations have shown that even in closely neighbouring skin snips the microfilarial densities may differ considerably, the mean microfilarial density of all the ethanol-fixed test snips was equal to that of the unfixed control snips. This confirms that the microfilariae in the fixed snips had been quantitatively detected with considerable accuracy.

Biopsies of the capsule of the nodules

The number of microfilariae could easily be assessed when the disintegrating fibres of the tissues were carefully pulled apart with needles after incubation in the enzyme solution.

4. CONCLUSIONS

This technique of digesting fixed nodules and biopsies provides several new possibilities for work in the field and in the laboratory. In epidemiological studies or even in drug trials nodules can now be collected, fixed and stored by teams which are not immediately

equipped to use the collagenase technique. The fixed nodules can then be sent to a central laboratory for digestion and evaluation under standardized conditions. This method could be of particular interest to the Onchocerciasis Control Programme in the Volta River Basin (OCP) where the process of aging of the adult worm population must be studied after the interruption of transmission.

Although the collagenase technique is the most precise quantitative skin snip technique, it is too laborious to replace the standard techniques for routine work in the field. However, it is of value in special cases, e.g. in a low-prevalence and low-intensity situation such as is likely to be found in the later stages of the OCP. At this stage, when more sensitive parasitological techniques are needed, "negative" biopsies in the field can now be fixed and re-examined later by the collagenase technique in a central laboratory.

RESUME

Il est possible d'isoler les Onchocerca volvulus adultes vivants et intacts à partir des nodules par digestion enzymatique du tissu humain environnant au moyen de collagénase. Cette technique exigeant habituellement des tissus frais, les problèmes de transport et la durée du stockage en empêchaient l'utilisation par les personnels de terrain. C'est pourquoi on a essayé d'effectuer la digestion sur des nodules onchocerciens fixés par l'éthanol, et on a étudié la faisabilité de l'évaluation qualitative et quantitative des vers adultes ainsi isolés. La technique de la collagénase a également été adaptée à l'évaluation de la densité de micro-filaires dans les biopsies fixées par l'éthanol.

Le processus de digestion était plus long avec le matériel fixé qu'avec du matériel frais. Toutefois, l'exposition prolongée à la solution enzymatique (pendant plusieurs jours) n'altérait pas la morphologie des vers ou des microfilaires fixés par rapport aux échantillons digérés sans fixation préalable.

Il a été conclu que cette technique de digestion des nodules et biopsies fixés ouvrait de nouvelles perspectives pour le travail sur le terrain et en laboratoire. Ainsi, lors des études épidémiologiques ou même pour les essais de médicaments, des équipes ne disposant pas au moment même du matériel nécessaire pour appliquer la technique de la collagénase peuvent recueillir, fixer et conserver les nodules. Les nodules fixés peuvent ensuite être expédiés à un laboratoire central qui effectuera la digestion et l'évaluation dans des conditions normalisées. Cette méthode pourrait être particulièrement intéressante pour le programme de lutte contre l'onchocercose dans le bassin de la Volta (OCP) où le processus de vieillissement de la population de vers adultes doit être étudié après l'interruption de la transmission.

La technique de la collagénase est la méthode la plus précise d'évaluation quantitative des biopsies cutanées, mais elle est trop compliquée pour remplacer les techniques standards dans le travail de routine sur le terrain. Elle peut toutefois présenter un intérêt dans des cas particuliers. Par exemple, dans une situation de faible prévalence et de faible intensité, comme il s'en rencontrera probablement lors des derniers stades du programme, les biopsies considérées comme "négatives" sur le terrain pourront être fixées et réexaminées plus tard par cette technique dans un laboratoire central.

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